033339/208804

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:
ELBAZ et al. : GROUP ART UNIT: 1653

SERIAL NO: 09/762,194

FILED: AUGUST 2, 1999 : EXAMINER: Robert A. WAX

FOR: NUCLEIC SEQUENCES CODING
FOR AN AT2 INTERACTING PROTEIN
INTERACTING WITH THE AT2 RECEPTOR
AND THEIR APPLICATIONS

DECLARATION UNDER 37 C.F.R. 1.132

HONORABLE COMMISSIONER OF PATENTS AND TRADEMARKS
WASHINGTON, D.C.

SIR:

Now comes Arthur Donny STROSBERG, who declares and states that:

1. I am a graduate of Sciences (Chemistry) and received my Doctorate degree in the year 1970.

2. I have been employed by CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE for 8 years as the Director Unit of Molecular Immuno-Pharmacology at INSTITUT COCHIN DE GENETIQUE MOLECULAIRE, in the field of molecular immuno-pharmacology.

3. I declare that I am experienced in the yeast two-hybrid method.

4. The yeast two-hybrid method is known in the art and was developed by Fields and Song in 1989 (Nature, 1989, 340, 245-246). This method enables not only the identification of interacting partners but also the characterization

of known interaction couples and even embodies the technological means to manipulate protein –protein interactions.

In the instant case, the modular properties of GAL4 transcription factor is exploited; more specifically, the instant method exploits the fact that the DNA-binding domain of GAL4 (DB) is incapable of activating transcription unless physically, but not necessary covalently associated with an activating domain (AD).

5. By using this method with the sequence encoding the cytoplasmic domain of the AT2 receptor fused to the DB of Gal 4 as a bait, a specific protein, named the AT2 interacting protein was characterized; this was confirmed both by the yeast two-hybrid method and *in vitro*; two chimeras, one containing the DNA-binding domain (DB) and one that contains an activation domain (AD) are co-transfected into an appropriate yeast strain. If the fusion partners (i.e. C-terminal fragment of the AT2 receptor and ATIP) interact, the DB and AD are brought into proximity and can activate transcription of reporter gene (here LacZ) (see annexed figure A).

6. In these conditions, it is not complicated for the man skilled in the art, to modify either the sequence encoding the C-terminal fragment of the AT2 receptor or the ATIP, in view to verify if said interaction is still present and therefore to use the different obtained systems as screening tools.

Knowing that it was easy for the man skilled in the art at the time the invention was made to prepare a two-hybrid system library, with different baits and preys, including sequences encoding either the C-terminal end of AT2 receptor or ATIP in a mutated form, it is not appropriate to describe and therefore claim specifically one or two mutations systems, the interest of the method being clearly in the use of a maximum screening systems and therefore in the preparation of a library comprising different two-hybrid systems. Such tools are very important in view of the action of ATIP when interacting with the C-terminal portion (cytoplasmic portion) of the AT2 receptor. (see figure B).

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the Application or any Patent issued thereon.

13/04/04
Date

(signature)

A. D. Strasberg
(Print or Type Name)

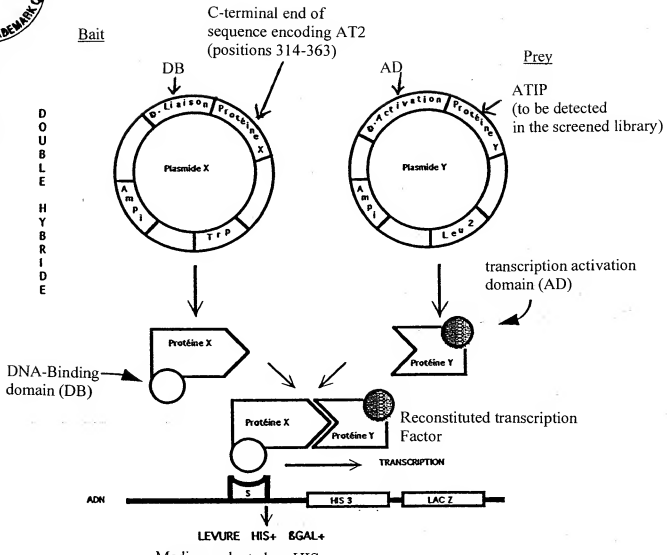

FIGURE A

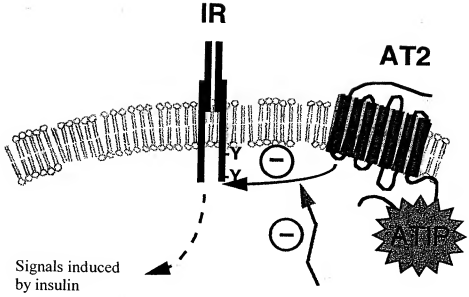

FIGURE B